REMARKS/ARGUMENTS

Claims 120-138, previously presented, are pending.

Claim 136 is amended, hereby, to correct a clerical error ("and" to "or") and to overcome the §112, ¶2, rejection of record, as explained below.

Claim 136 was rejected under 35 USC 112, ¶2. Reconsideration is requested in view of the instant amendment to the claim.

Claim 136 is amended, hereby, as suggested by the Examiner in order to overcome the rejection. That is, the language "alcoholic solution" in claim 136, line 4, is modified to "the alcoholic portion of the alcoholic solution." Accordingly, withdrawal of the rejection under §112, ¶2, appears in order. Applicant wishes to thank the Examiner for helpfully suggesting alternative claim language for overcoming the rejection.

Claims 120-138 were rejected under 35 USC 103(a) based on the combined teachings of Henco (US 5,057,426), Little (US 5,075,430), *International Dictionary of Medicine and Biology*, Vol. 1, 1986 (New York) page 522 (International Dictionary), and *Nucleic Acid Hybridisation - A Practical Approach*, 1985 (Washington D.C.) pages 64, 65, and 235 (Hames). Reconsideration is requested.

As broadly claimed (in claim 120) the instant invention is directed to a process for the isolation and purification of nucleic acids from cells using two separation/purification stages – (i) and (ii), as recited in claim 120.

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Purification stage (i) includes steps (a) and (b). According to the first step (a) the cells containing nucleic acids are digested, the resulting cell debris removed, and the nucleic acids from the cells treated with a low ionic strength buffer solution (first buffer solution) in the presence of an anionic exchanger, such that the nucleic acids are adsorbed on the surface of the anionic exchanger.

In stage (i), step (b), the nucleic acids are desorbed from the anion exchanger by applying a second buffer solution having a higher ionic strength than the first buffer solution. Accordingly, once-purified nucleic acids (i.e., in this second buffer) are produced by stage (i).

Purification stage (ii) includes steps (c) and (d).

According to stage (ii) step (c), the one-purified (protein-free) nucleic acids in the second buffer solution [from stage (i)] are applied and, so, adsorbed onto the surface of a mineral support.

In stage (ii), step (d), the nucleic acids are desorbed (i.e., eluted) from the mineral support by applying, either, water or a third buffer solution having a lower ionic strength than the second buffer solution.

Accordingly, twice-purified nucleic acids are produced. Preferentially, according to claim 125, a washing step is introduced in stage (ii), between steps (c) and (d), by applying an aqueous alcoholic solution.

More preferentially, according to claim 136, the alcoholic solution includes 1 to 7 M sodium perchlorate, 1 to 7 M guanidine hydrochloride, 1 to 6 M sodium iodide, or 1 M sodium chloride in 20 % ethanol, propanol, iso-propanol, butanol, poly(ethylene glycol), or mixture thereof.

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The salts sodium perchlorate, guanidine hydrochloride, and sodium iodide belong to the group of chaotropic substances or chaotropes according to the so-called "Hofmeister series," as defined, e.g., in *On-line Medical Dictionary* (URL http://cancerweb.ncl.ac.uk/omd/)(copy attached):

> Hofmeister series
> <chemistry> The series of cations Magnesium, Calcium, Sr2+, Ba2+, Li+, Na+, K+, Rb+, Cs+, and of anions citrate3-, tartrate2-, SO42-, acetate-, NO3-, ClO3-, I-, CNS- (among others).
> Each series is arranged in order of decreasing ability to: 1) precipitate the dispersed substance of lyophilic soltions; 2) "salt out" organic substances (e.g., aniline, ethyl acetate) from aqueous solutions; or 3) inhibit the swelling of gels.
> These effects, among other related ones, are ascribable to the abstraction and binding of water by these ions (i.e., hydration), which also decreases in the orders given, so that (in the monovalent cation series) Li+, with the smallest crystal radius, has the largest hydrated radius, and vice versa for Cs+.
> Synonym: lyotropic series.

The counterpart of these chaotropes are the so-called kosmotropes.

The term 'chaotrope' (disorder-maker) originally denoted a stabilizing solute for proteins and membranes. Conversely, the term 'kosmotrope' (order-maker) originally denoted a destabilizing solute for proteins and membranes. Later, they were used to denote the apparently correlating property of increasing the structuring of water (chaotrope) and decreasing the structuring of water.

Large, singly-charged ions with low charge density (e.g., $H_2PO_4^-$, HSO_4^-, HCO_3^-, I^-, Cl^-, NO_3^-, NH_4^+, Cs^+, K^+) which exhibit weaker interactions with water than water with itself, are chaotropes. On the other hand, small or multiply-charged ions, with high charge density (e.g., SO_4^{2-}, HPO_4^{2-}, Mg^{2+}, Ca^{2+}, Li^+, Na^+, H^+, OH^- and HPO_4^{2-}), which exhibit stronger interactions with water molecules than water with itself, are kosmotropes.

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From the above explanation it is readily seen that sodium chloride cannot be classified as a "classic" chaotropic substance. On the contrary, sodium chloride appears better classified as a kosmotrope, in the sense of the Hofmeister series.

According to the statement of rejection, Henco's allegedly inherent use of urea as a chaotropic compound apparently provides motivation for combining Henco and Little, in the manner alleged in the statement of rejection.

Henco is concerned with a method for the separation of long-chain nucleic acids from biological samples. The method involves applying lysed samples, and fixing the long-chain nucleic acids in the samples, to a porous matrix, washing the porous matrix to separate other substances from the fixed long-chain nucleic acids, and removing the fixed long-chain nucleic acids from the porous matrix.

In connection with this purification, Henco teaches the use of urea for lysing cells, bacteria, and viruses, as an initial step of the separation method. For example:

Column 8, lines 59 to 63:

> *The method according to the invention utilizes the described porous matrix by lysing the CMV viruses in situ by addition of urea, detergent and buffer, whereupon the DNA (130 to 150.times.10.sup.6 Dalton) is released.*

Column 12, lines 33 to 38 [Example 2]:

> *Upon simultaneous addition of 4 M of urea, the DNA of the phages is released and, by means of another filtration through the cartridge, specifically adsorbed on the anion exchanger. Then the cartridge is washed with 0.8 M NaCl, 50 mM Tris-HCl buffe, pH 7.5, 1mM EDTA, and the DNA is eluted with about 1 ml of 1.2 M NaCl, 50 mM Tris-HCl buffer, pH 7.5, 1 mM EDTA.*

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Column 12, lines 63 to 69 [Example 3]:

> *...and the phage pellet is dissolved in 20 µl of 10 mM Tris, 1 mM EDTA, pH 7.5. Another part by volume of extraction buffer (2% Triton X-100®, 7 M urea, 100 mM EDTA, pH 7.5) is added, and the mixture is heated at 50 °C for 15 minutes to release the single-stranded DNA.*

Column 13, lines 14 to 24 [Example 4]:

> *The isolation of cellular DNA from sperm is carried out as follows:*
>
> *One hundred µl of sperm are suspended in 1 ml of 500 mM NaCl, 10 mM EDTA 40 mM DTE, 10 mM Tris-HCl buffer, pH 7.5, 1% Triton, 4 M urea and 20 µg/ml of proteinase K and incubated at 37 °C for 2 hours. After centrifugation at about 5000 g for 5 minutes, the supernatant is passed through the separating gel in a cartridge. The flow velocity of the supernatant through the cartridge is about 1 ml/min.*

Column 13, lines 64 to 68 [Example 7]:

> *The preparation of CMV (cytomegalovirus) DNA from urine is carried out as follows:*
>
> *CMV viruses are lysed in situ upon addition of 4 M urea, 1% Triton, 500 mM NaCl, 50 mM Tris-HCl buffer, pH 7.5*

Henco teachings are limited to using urea, exclusively, as a lysing agent in the initial steps of the separation method. The reference does not even hint at using urea for anything else or in any other step of the separation method. Likewise, there is no hint to use urea as an elution or washing buffer, i.e., for the purpose of washing or eluting nucleic acids adsorbed on a matrix. On the contrary, Henco discloses that the addition of urea to the loading buffer (i.e., before the adsorption/binding of the nucleic acids) has no effect on the binding behavior of the long chain DNA (column 7, lines 8 to 11):

> *The addition of urea to the loading buffer does not affect the binding behavior of the long-chain DNA, while it optimizes the separation efficiency with respect to proteins.*

The only advantageous effect of the addition of urea results in a better separation efficiency with regard to the proteins. This is of no help at all with regard to the solution of the problem underlying the presently claimed invention.

According to the presently claimed invention, the product of stage (i), i.e., the starting material for stage (ii), is already once-purified, protein-free nucleic acids. Nothing, whatsoever, in Henco and Little, together, suggests arranging Henco and Little in tandem, as alleged in the rejection, i.e., so that Little starts with purified, rather than a new source of, nucleic acids. Looking at the matter another way, Henco's use of urea as a lysing agent would not have led one skilled in the art to use chaotropic substances in a buffer to wash nucleic acids which are bound on a matrix.

Little is concerned with a process for the purification of plasmid and other DNA, both single-stranded and double-stranded, by immobilizing the DNA onto diatomaceous earth in the presence of a chaotropic agent and eluting the DNA with water or low salt buffer.

The Little invention is based on the finding that diatomaceous earth is useful for the purification of DNA by immobilizing the DNA onto the diatomaceous earth particles in the presence of a chaotropic agent, and elution of the DNA with water or low salt buffer. More particularly, Little discloses a process for the purification of plasmid DNA comprising the following steps:

a) *immobilizing the DNA onto diatomaceous earth in the presence of a chaotropic agent;*

b) *washing the resulting diatomaceous earth-bound DNA with an alcohol-containing buffer;*

c) *removing the alcohol-containing buffer; and*

d) *eluting the DNA in a low salt buffer or in water.*

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According to Little, a chaotropic substance is to be understood as a

> *... substance that enhances the partitioning of nondollar molecules from a nonaqueous to an aqueous phase as a result of the disruptive effect that the substance has on the structure of water. Examples of chaotropic agents include sodium iodide, sodium perchlorate and sodium trichloroacetate.*

[column 3, lines 36 to 43] Sodium chloride forms no part of the mentioned chaotropic sodium salts. It is no doubt true that the chaotropic substance is primarily used to bind the nucleic acid on the diatomaceous earth matrix.

However, Little also requires use of a chaotropic binding buffer and a 50% ethanol buffer [column 4, lines 21 to 35]:

> *An example of an alcohol-containing wash buffer comprises: 20.0 mM Tris-Cl pH 7.5, 20 mM EDTA, 0.4 M NaCl, and 50% v/v ethanol. This buffer will be abbreviated herein as "50% ethanol buffer" or "50% washing buffer".*
>
> *In order to lower the RNA and protein concentration in plasmid lysates, it is necessary to perform a sufficient number of washes using the chaotrope binding buffer and the 50% washing buffer. The amount of RNA and protein remaining is indirectly proportional to the number of volume washes performed on the diatomaceous earth pellet, membrane or column. Generally, about three washes of each buffer is sufficient to lower the RNA and protein concentrations to acceptable levels.*

Accordingly, the washing steps using the chaotropic buffer, on one hand, and the washing buffer, on the other, are necessary to lower the RNA and protein concentrations in plasmic lysates. As in Henco, this step is necessary for starting from raw biological samples and not once-purified nucleic acids.

Moreover, Little's objective, with regard to the elution of the DNA, was to optimize the yield of DNA to be recovered [Little column 4, lines 36 to 46]:

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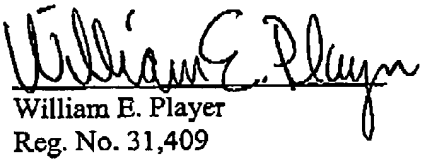
The efficiency of release of immobilized DNA from the diatomaceous earth pellet, membrane or column will be proportional to the ratio of the volume of low-salt buffer or water added to the volume of the pellet, membrane or column. Thus, with a 5 µl diatomaceous earth pellet, for example, 5 µl of buffer or water (1 volume) will extract about 50% of the DNA. Likewise, 10 volumes of buffer or water added per volume of pellet will permit the recovery of >90% of the DNA. However, it should be kept in mind that the more buffer or water added, the more dilute the eluted DNA.

The rejection tries to take the word "urea" completely out of context of the teaching of Henco to find an inherent feature (which is of no meaning for the use disclosed by Henco). The rejection relies on a torturous combination of prior art teaching using impermissible hindsight reconstruction to meet the limitations of the present claims.

Favorable action is requested.

Respectfully submitted,

JACOBSON HOLMAN PLLC

By: 

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Hofmeister series

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Synonym: lyotropic series.

(05 Mar 2000)

Previous: Hofmeister, Franz von, Hofmeister gastrectomy, Hofmeister-Polya anastomosis
Next: Hofmeister's operation, hog, hogback, Hogben, Lawrence, Hogben number

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